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REACTIVATION OF AGED ORGANOPHOSPHORUS INHIBITED ACETYLCHOLINESTERASE

Annual Summary Report

David R. Dalton

31 August 1986

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-C-5195

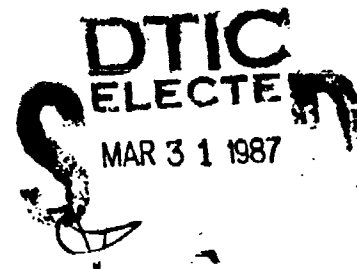
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Philadelphia, Pennsylvania 19122

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Summary:

→ The purpose of this work is to prepare derivatives of 2-PAM that are substituted in the 3-position by groups capable of bonding to, or liganding with, the oxygen on the phosphorus of aged, poisoned (by phosphorylation or phosphonylation) acetylcholinesterase. The derivatives suggested for preparation are designed to facilitate cleavage of the bond between phosphorus and the oxygen of the serine in the active site of the poisoned acetylcholinesterase and to thus regenerate native enzyme.

The major effort in this first year has been development of a general route to these previously unknown oximes. That route, beginning with derivatives of 3-aminocrotonate and acrolein, has now been defined. Additionally, using the successful route, one compound has been prepared and submitted (i.e., ethyl pyridine-3-acetate 2-carboxaldehyde oxime methiodide, BL19566), another prepared (i.e., t-butyl pyridine-3-carboxylate 2-carboxaldehyde oxime methiodide) but not submitted (because the methochloride, not the methiodide as originally anticipated, is desired and anion exchange has not yet succeeded), and significant quantities of intermediates for other proposed compounds have been generated.

We conclude that the synthesis of most of the initially proposed compounds will be accomplished but that our original schedule was unrealistically optimistic.

↓
Keywords: Pyridine Oximes

Statement of the Problem:

It has been argued¹ that failure to regenerate native acetylcholinesterase from that which has been poisoned with derivatives of methylphosphonic acid (e.g., soman, sarin, and VX) through less-than-immediate treatment with 2-PAM (or its relatives such as toxogonin) may be due, in part, to ageing. By this is meant that, of the two ester groups extant in the phosphorylated (poisoned) acetylcholinesterase preferential hydrolysis of the smaller group occurs and the blocked serine residue in the poisoned cholinesterase is retained. The aged, poisoned enzyme is now refractory to 2-PAM, etc., treatment because the leaving group ability of the phosphonic acid derivative bearing only one ester (at serine) is significantly poorer than that with two.

As immediate treatment in the field cannot be universally warranted and as there is some evidence² that a "depot" of organophosphorus poison may be extant even after treatment, an effort to provide compounds effective in the regeneration of acetylcholinesterase from aged poisoned enzyme is recognized.

The work undertaken addresses this problem.

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Foreword:

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Table of Contents

1.	Background	6
2.	The Syntheses to Date	9
3.	Discussion: Series a	11
4.	Experimental Section: Series a	13
5.	Discussion: Series b	16
6.	Experimental Section: Series b	21
7.	Discussion: Series c	27
8.	Experimental Section: Series c	29
9.	References	33

Background:

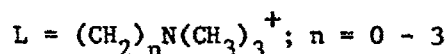
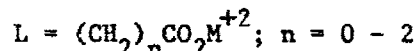
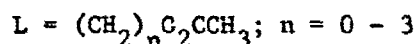
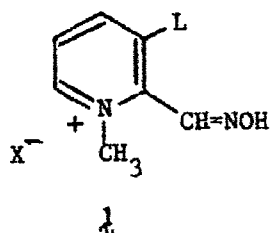
The general consensus regarding the active site of the acetylcholinesterases which are affected by organophosphorus poisons is that they are similar to the active site of trypsin and other serine proteases.¹ Thus, the traditional "charge-relay-pathway"³ is assumed operational for acetylcholinesterase as it is for serine proteases. However, there is some debate on the details of the process by which the serine proteases accomplish ester hydrolysis⁴ and, indeed, the particulars of the structure of acetylcholinesterase (electric-eel enzyme) have not yet been fully reported⁵ - although it is generally agreed that a serine residue occupies the active site. Further, a recent study of the proton inventory during the deacetylation of phenyl acetate (as an analogue of natural substrate) by electric-eel enzyme, reveals that catalysis by a single protonic site, rather than multiple sites as in other serine proteases, obtains.⁶ Thus, whatever may eventually turn out to be correct for the pathway for ester hydrolysis by the serine proteases in general, may not be analogous to what is occurring with acetylcholinesterase.

Despite these general uncertainties, it is currently believed that phosphorylation (or phosphonylation) of acetylcholinesterase at serine blocks hydrolysis of acetylcholine. Regeneration of enzyme can be effected by oxime therapy in which, presumably, the therapeutic agent reacts with phosphorylated (phosphonylated) acetylcholinesterase and liberates native acetylcholinesterase and phosphorylated (phosphonylated) oxime. The reaction is facilitated by using an oxime bearing a positive charge, as it appears that there is a negative site on the enzyme proximate to the phosphorylated (phosphonylated) serine which coordinates with the positive charge and promotes the desired O-P bond cleavage.

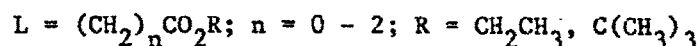
In the absence of therapeutic oxime, hydrolysis of a different phosphorus-oxygen ligand appears to occur, the enzyme is said to have aged, and regeneration is much more difficult. We presume this is because the resulting anion (or its conjugate acid) is less readily attacked at phosphorus by the oxime.

To promote regeneration of the acetylcholinesterase from such aged phosphorylated (phosphonylated) enzyme either the oxygen having lost its alkyl group can be realkylated or another substitution at oxygen effected. Given the chemistry of phosphonic acids and their known resistance to substitution⁷ the former is less likely than the latter. Hence, methods for reaction at oxygen of materials likely to survive the environment long enough to consummate the desired transformation were sought. Three are proposed. First, acetylation or complexation of the unsubstituted oxygen with the positive carbon of the carbonyl of an ester is considered. Second, coordination of the (negative) oxygen remaining after cleavage of the alkyl group with a metal ion, known to facilitate dephosphorylation⁸ is suggested. Finally, electrostatic interaction between a positively charged nitrogen and the (presumably) negative oxygen on phosphorous is proposed.

The family of compounds to be prepared is thus generally represented as 1. (below).



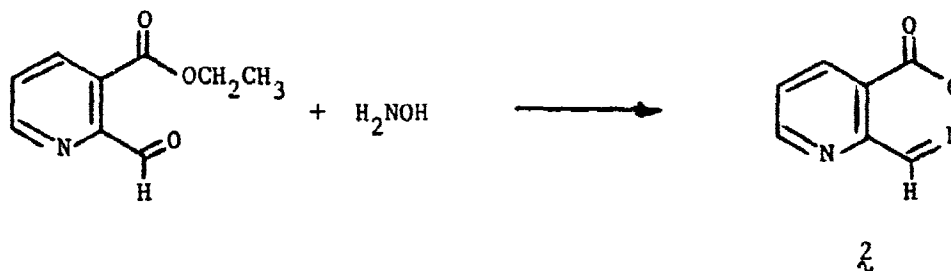
additionally



X = I, Cl (originally unspecified)

There is little evidence that substitution at C-3 (L, above) plays any role in the effectiveness of pyridinium oximes to act as desired. Indeed, it appears that only 3-methyl-2-PAM (1, L = CH₃) has been compared to 2-PAM (1, L = H) itself and the former was found to be somewhat less effective.⁸

Substitution at oxygen of phosphonic acids has been achieved by alkylation with diazo compounds, alkyl iodides, and methyl sulfate.⁷ There is some evidence that oxiranes might also be successfully used.⁷ However, we now believe (based on some of our findings herein) as we had only originally suspected, that such reactive alkylating groups at C-3 will not themselves survive to interact with the aged poisoned acetylcholinesterase. Thus, even preparation of the ester 1, L = CO₂CH₂CH₃, is intruded upon by its apparent self-destruction to form 2 (Equation 1).

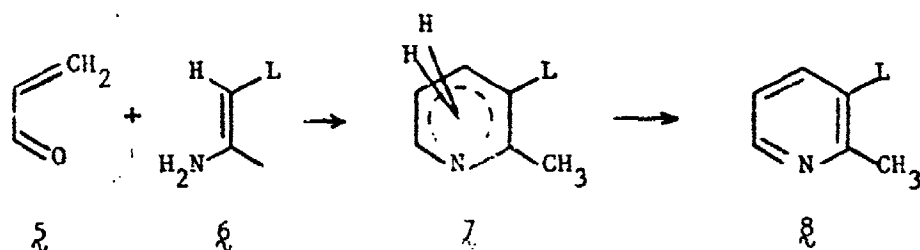
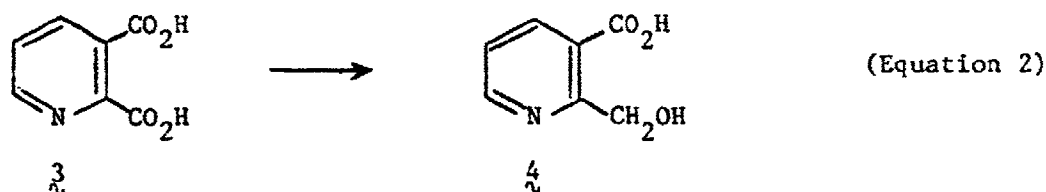


(Equation 1)

Other similarly or more reactive groups, e.g., $L = CH_2Br(I)$, $L =$ oxirane, $L = CHN_2$, etc. would thus probably suffer the same or similar fates.

However, acylation of oxygen on phosphorus⁹ or even formation of a tetrahedral intermediate (i.e., the intermediate in $O \rightarrow O$ acyl transfer) with the carbon of the carbonyl group should be more facile than alkylation and such materials might be stable enough to survive preparation. Further, the metal salts (I , $L = (CH_2)_nCO_2M^{2+}$), known to facilitate phosphate ester hydrolysis,⁸ should be capable of preparation as should the bisquaternary salts I , $L = (CH_2)_nN(CH_3)_3^+$.

While the desired compounds had not hitherto been prepared, two general routes were available to the pyridine nucleus. First, reduction of pyridine-2,3-dicarboxylic acid (quinolinic acid), 3 , to 2-hydroxymethyl pyridine-3-carboxylic acid, 4 (or the corresponding lactone), had been reported (Equation 2).¹⁰ This material could reasonably be expected to provide a starting point for further functionalization and thus elaboration to the desired compounds. Second, the well known condensation reaction between acrolein (or its derivatives), 5 , and derivatives of 3-aminocrotonic acid, 6 , could be employed (Equation 3).¹¹



(Equation 3)

In the event, the second route, avoiding the difficulties inherent in separation of closely similar analogues and using readily available starting materials was chosen. The details of conversion of the 3-substituted 2-methylpyridines to oximes followed, for the most part, reactions for which significant precedent exists.

The Syntheses to Date:

(1) The Initial Condensation Reaction (Equation 3):

Satisfactory results in the initial condensation reaction, Equation 3, have been obtained only for $L = \text{CO}_2\text{CH}_2\text{CH}_3$, $L = \text{CO}_2\text{C}(\text{CH}_3)_3$ and $L = \text{CN}$ (for the latter only through to the mixture of dihydropyridines, γ). The first two were most fully explored as the latter was primarily directed toward eventual production of amino groups at C-3 and similar compounds were readily available from $L = \text{CO}_2\text{CH}_2\text{CH}_3$. In the same vein, early studies using δ , $L = \text{CONH}_2$, were abandoned. Extensive efforts were expended in examination of the oxidative conversion of the mixture γ to the aromatic δ ($L = \text{CO}_2\text{CH}_2\text{CH}_3$). As the preparation of γ is accompanied by large quantities of acrolein polymer, two choices were available and both were explored. First, oxidation of the dihydropyridine mixture γ could be attempted after its separation from polymer accompanying its formation or, second, oxidation to the pyridine could be effected prior to purification.

Fractional vacuum distillation of the crude reaction mixture containing the ethyl esters γ ($L = \text{CO}_2\text{CH}_2\text{CH}_3$) resulted in yields as high as 50% of theory of crude dihydropyridines. Small amounts of aminocrotonate could also be recovered but the remainder of the material appears to be trapped as acrolein copolymer and is intractable. The crude reaction mixture contains some pyridine (gas chromatography) and the dihydropyridine distillation is apparently accompanied by additional oxidation to the pyridine. Steam distillation and chromatographic methods (including hplc but not preparative gc) were less satisfactory on large scale reactions although, on a small scale, comparable yields of crude material were obtained.

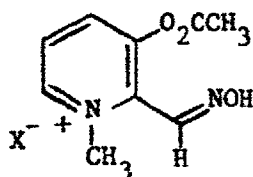
Sulfur oxidation, air oxidation (catalyzed and uncatalyzed), and oxidation with nitric acid all successfully converted the crude mixture into pyridine and, although yields from the sulfur method were slightly higher than those by nitric acid oxidation, work-up was far more tedious than when nitric acid was used. Air oxidation, the most gentle method, gave the lowest yields.

Oxidation prior to purification was only successful when air was used as the oxidant. Modest yields result and one of the dihydropyridines (γ) is clearly more easily oxidized than other(s). However, only one purification step is now required and, for $L = \text{CO}_2\text{C}(\text{CH}_3)_3$ this appears to be the method of choice. Thus, depending on the precise methods used (vide infra), for $L = \text{CO}_2\text{CH}_2\text{CH}_3$, reproducible yields of 40 - 50% of δ can now be routinely obtained and for $L = \text{CO}_2\text{C}(\text{CH}_3)_3$, yields of the order of 20 - 30% of δ are normal. It is possible that these reactions might be further optimized but, currently, we do not intend to expend the effort.

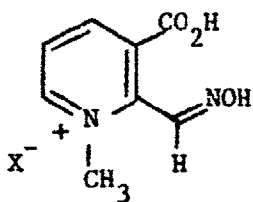
Details of the synthetic procedures are discussed subsequent to the schemes that follow.

(2) Compounds Prepared and in Preparation:

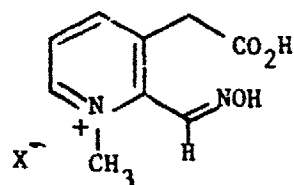
Three series of compounds (of the four series outlined in the original proposal) have been examined in the first year. All are N-methyl-2-pyridinium carboxaldehyde oximes and are distinguished as Series a, phenolic oxygen at C-3 (i.e., zero carbons at C-3); Series b, one carbon at C-3 and, Series c, two carbons at C-3. These are shown in the Schemes that follow as the acetylated phenol (Series a), and the carboxylic acids in Series b and c, respectively. The details of the syntheses are discussed subsequent to the Schemes.



Series a, no carbons at C-3



Series b, one carbon at C-3



Series c, two carbons at C-3

Discussion:

Series a - Scheme 1:

Initially, the diacetate, 10, of commercially available 3-hydroxy-2-(hydroxymethyl)pyridine, 9, was prepared in the hope that partial hydrolysis to 3-acetoxy-2-(hydroxymethyl)pyridine could be effected. Clearly, should such a process prove possible, oxidation of the remaining hydroxyl to the aldehyde, formation of the oxime, and methylation would yield the desired product. In the event, however, only partial hydrolysis did occur but, in low yield, the phenolic acetate appeared to undergo hydrolysis preferentially and, of the monoacetate isolated, (ca 5% yield) none of the desired product was present. The reasons for the poor yield of monoacetate obtained, even in the presence of a limited amount of base, were not investigated as the facile oxidation of the benzylic hydroxyl of 9 to the corresponding aldehyde 11 was readily consummated¹² and it was presumed that acetylation of the phenolic hydroxyl of the aldehyde would serve as well.

Interestingly, attempted acetylation of 11 to the monoacetate 12 was frustrated by the apparent fragility of the latter. Thus, although even at short reaction times the aldehyde clearly reacted (gc) with acetic anhydride and all of the starting material was consumed, only the starting material, 11, and diacetate, 10, the product of an apparent Cannizzaro redox reaction, followed by acetylation, could be obtained. The oxidation product of the Cannizzaro reaction - presumably the corresponding carboxylic acid - was not isolated.

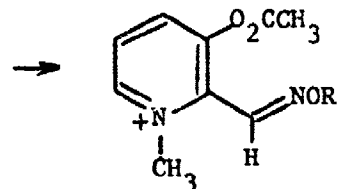
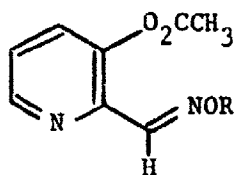
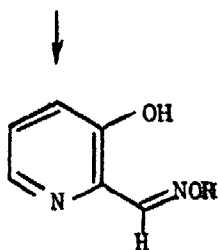
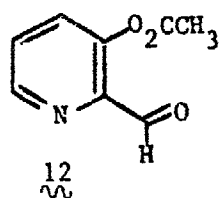
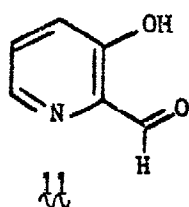
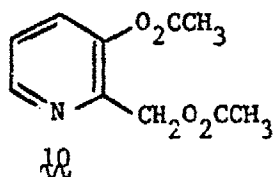
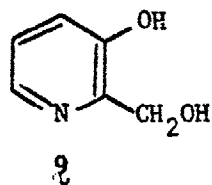
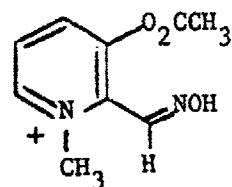
Thus, the hydroxyaldehyde, 11, was converted to the known oxime, 13i.¹³ Again, the diacetate, 14i, was prepared and, again, partial hydrolysis could not be effected to the corresponding 3-acetoxy-2-oxime. Only phenolic material resulted. While it is true that this may indicate preferential hydrolysis of the phenolic acetate, we suspect that even if the oximino-acetate is undergoing hydrolysis first, isolation of the desired material may be intruded upon by facile O → O acyl migration. This was not, however, investigated. Instead, O-benzylhydroxylamine was utilized to prepare the corresponding phenolic O-benzylloxime, 13ii.

Precedent for O-debenzylation by hydrogenolysis¹⁴ led us to believe that formation of the acetate 14ii, followed by careful hydrogenolysis might lead to the desired oxime. Under a variety of conditions however, either no reduction occurred or reduction to the corresponding O-acetylamine, accompanied by O → N acyl migration leading to a phenolic amide intruded. Thus, it was clear that a more easily removed group was needed. We thus turned our attention to the use of O-t-butyldimethylsilyl hydroxylamine.¹⁵

O-t-Butyldimethylsilyl hydroxylamine was prepared and allowed to react with the aldehyde 11. The corresponding oxime 13iii was obtained and acetylation to 14iii effected. Methylation to the corresponding methiodide of 13iii with methyl iodide has also succeeded. Acetylation of 14iii with acetic anhydride to 15i was also successful. However, treatment of the silylated oxime with a variety of fluoride anion sources (for desilylation) has not yet provided the desired oxime. However, this process, which should prove most general for other acetates desired as well as this specific acetate is being pursued.

Series a

SCHEME 1



13 i, R = H

13 ii, R = $\text{CH}_2\text{C}_6\text{H}_5$

13 iii, R = $\text{Si}(\text{CH}_3)_2\text{t-Bu}$

14 i, R = COCH_3

14 ii, R = $\text{CH}_2\text{C}_6\text{H}_5$

14 iii, R = $\text{Si}(\text{CH}_3)_2\text{t-Bu}$

15 i, R = $\text{Si}(\text{CH}_3)_2\text{t-Bu}$

Experimental Section: Series a

3-Hydroxy-2-(hydroxymethyl)pyridine diacetate (10): 3-Hydroxy-2-(hydroxymethyl)pyridine hydrochloride, 9, (Aldrich, 5.0 g, 30 mmol) and sodium acetate (5.0 g, 60 mmol) were dissolved in acetic anhydride (150 mL) and the solution refluxed for 12 hr. On cooling to room temperature, the precipitated sodium chloride was removed by filtration and the filtrate was concentrated, in vacuo, to a yellow oil which was dissolved in a minimum amount of water. The aqueous solution was continuously extracted with chloroform and then concentrated to obtain 10 (5.6 g, 27 mmol, 90%) as a yellow oil. IR (neat) 1720 cm^{-1} (C=O). ^1H NMR, 2.08 (3H, s), 2.20 (3H, s), 5.22 (2H, s), 7.28 (1H, dd), 7.50 (1H, dd), 8.48 (1H, dd).

Attempted preparation of 3-acetoxy-2-(hydroxymethyl)pyridine: To a 1:1 aqueous ethanolic solution (50 mL) of 3-hydroxy-2-(hydroxymethyl)pyridine diacetate (10) (2.09 g, 10 mmol) there was added, over 10 min, a solution of potassium hydroxide (560 mg, 10 mmol) in aqueous ethanol (20 mL) with stirring. After 45 min at room temperature, the reaction was acidified with 6N HCl and rebasified with saturated aqueous bicarbonate. The aqueous solution was continuously extracted with ether, the organic phase dried (sodium sulfate), filtered, and evaporated to dryness in vacuo to yield a dark residue. TLC (Analtec, silica gel, 2000, 1:1 CHCl_3 -EtOAc) yielded 2-acetoxymethyl-2-hydroxypyridine (8.6 mg, 5%, mp $126\text{--}128^\circ$). IR (KBr) 1740 cm^{-1} (C=O), ^1H NMR 2.1 (3H, s), 5.2 (2H, s) 7.2 (3H, m), 8.13 (2H, m).

3-Hydroxy-2-(carboxaldehyde)pyridine (11): 3-Hydroxy-2-(hydroxymethyl)pyridine hydrochloride (9) (1.0 g, 6.2 mmol) and manganese dioxide (2.0 g, 0.23 mol) were suspended in dry chloroform (20 mL) and stirred at room temperature (drying tube) for 6-8 hr. Progress of the reaction was monitored by tlc (1:1 ether:hexane). When the starting material had disappeared, the solids were removed by filtration and the solvent removed at reduced pressure. The resulting solids were transferred to a soxhlet thimble and extracted with ether for 24 hr and the ether concentrated. The resulting crystals were recrystallized from hexane to yield 430 mg, 3.47 mmol, 56 %, mp $78\text{--}79^\circ$ (lit.¹⁷ $78\text{--}79^\circ$) of 11. IR (KBr) 1685 cm^{-1} (C=O), ^1H NMR 7.35 (2H, m), 8.30 (1H, m), 10.6 (1H, s).

3-Hydroxy-2-(carboxaldehyde)pyridine oxime (13i): 3-Hydroxy-2-(hydroxymethyl)pyridine hydrochloride (9) (16.2 g, 0.1 mol) and manganese dioxide (8.7 g, 0.1 mol) in absolute ethanol (200 mL) was heated with stirring to reflux and sulfuric acid (10.2 g, 0.1 mol) in ethanol (50 mL) was added over a period of 0.5 hr. The mixture was refluxed for 1 hr longer, cooled, and filtered. The dark yellow solution was diluted with water (200 mL) and manganese carbonate was precipitated by adding excess sodium bicarbonate (solid). The filtrate was extracted with ether (1 x 400 mL and 2 x 150 mL, and the combined ether extracts were washed with 3.7% aq. HCl (4 x 25 mL portions). Ethanol was removed (in vacuo) from the acidic extracts. The aqueous acid was then treated with sodium acetate (15 g, 0.18 mol) and hydroxylamine hydrochloride (14 g, 0.2 mol) and the reaction mixture warmed at 100° for 10 min. On cooling at 0° , the product oxime (13) crystallized, 2.1 g, 15.2 mmol, 15.2%, mp $175\text{--}176^\circ$ (lit.^{17,18} $175\text{--}176^\circ$). IR (KBr) 1425 cm^{-1} (C=N). ^1H NMR 7.32 (2H, m), 8.16 (1H, m), 8.22 (1H, s), 10.3 (1H, s). Subsequent preparations occasionally yielded a second isomer

which is related to the first as Z/E isomers (presumably). This isomer, mp 188° has not, apparently, been isolated before. IR (KBr) 1480 cm⁻¹, ¹H NMR 7.82 (1H, m), 8.20 (2H, m), 8.47 (1H, s).

Attempted preparation of 3-acetoxypyridine-2-carboxaldehyde (12):

(a) 3-Hydroxy-2-(carboxaldehyde)pyridine, 11, (2.0 g, 16.2 mmol) in acetic anhydride (30 mL) containing one drop of acetyl chloride was refluxed for 6 hr. On cooling, the acetic anhydride was removed at reduced pressure to yield 3-acetoxy-2-(carboxaldehyde)pyridine diacetate (1) (3.91 g, 14.6 mmol, 90%). IR (neat) 1790 cm⁻¹ (ester C=O), ¹H NMR 2.10 (6H, s), 2.31 (3H, s), 7.36 (2H, m), 7.94 (1H, s), 8.51 (1H, m).

(b) 3-Hydroxy-2-(carboxaldehyde)pyridine, 11, (2.0 g, 16.2 mmol) and acetic anhydride (1.82 g, 17.9 mmol) in anhydrous ether (30 mL) was heated at reflux while the reaction was monitored by gc (1.2% OV-17, He, 20 mL/min, 80-260 @ 200/min, inj. at 150°). When the starting material had disappeared the solvent was removed at reduced pressure and there was obtained, as a residue, 3-hydroxy-2-(hydroxymethyl)pyridine diacetate (10), 1.53 g, 7.32 mmol, 45%.

3-Hydroxy-2-(carboxaldehyde)pyridine O-benzyloxime (13ii): 3-Hydroxy-2-(hydroxymethyl)pyridine, 9, (16.2 g, 0.1 mol) and manganese dioxide (8.7 g, 0.1 mol) in absolute ethanol (200 mL) was heated, with stirring, at reflux while sulfuric acid (10.2 g, 0.1 mol) in ethanol (50 mL) was added over a period of 0.5 hr. The mixture was refluxed for another hr and, after cooling to room temperature and filtration, the dark yellow solution was diluted with water (200 mL) and manganese carbonate was precipitated by addition of excess sodium bicarbonate. Filtration was followed by extraction of the filtrate with ether (1 x 400 mL and 2 x 150 mL) and the combined ether extracts were washed with 3.7 % HCl (4 x 25 mL, 0.1 mol). The acidic extracts were combined and treated with sodium acetate (15 g, 0.18 mol) and O-benzylhydroxylamine hydrochloride (37 g, 0.2 mol) and the resulting solution was warmed on the steam bath for 20 min. On cooling to room temperature the O-benzyloxime (9.6 g, 42 mmol, 42%) 13ii, crystallized, mp 72-73° IR (KBr) 1450 cm⁻¹ (C=N). ¹H NMR 5.16 (2H, s), 7.10 (2H, m), 7.34 (5H, s), 8.12 (1H, m), 8.35 (1H, s), 9.65 (1H, s)

3-Hydroxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime (13iii): To a solution of 3-hydroxy-2-(carboxaldehyde)pyridine, 11, (1.0 g, 8.1 mmol) in dry chloroform (25 mL) was added O-t-butyldimethylsilyl hydroxylamine (1.2 g, 8.15 mmol) and activated 5A molecular sieves. The reaction mixture was stirred at room temperature under nitrogen and its progress was monitored by tlc (1:1 hexane:ether). Upon completion (ca. 48 hr), additional dry chloroform was added and the sieves removed by filtration. The solvent was removed at reduced pressure and the product was purified by preparative tlc using 1:1 hexane:ether as eluant to yield 1.27 g, 5.03 mmol, 62% of the desired oxime 13iii, mp 163-164 (dec). IR (1450 cm⁻¹) (KBr). ¹H NMR 0.98 (9H, s), 7.20 (2H, m), 8.18 (1H, m), 8.48 (1H, s).

3-Hydroxy-2-(carboxaldehyde)pyridine oxime diacetate (14i): 3-Hydroxy-2-(carboxaldehyde)pyridine oxime, 13i, mp 175-176° (1.0 g, 7.2 mmol), triethylamine (655.2 mg, 7.2 mmol) and acetic anhydride (1.92 g, 14.4 mmol) in ether (30 mL) was stirred at 0° for 6 hr. The reaction mixture was concentrated in vacuo and the residue was dissolved in a minimum amount of water and extracted with chloroform. The chloroform extract was dried by filtration and concentrated to yield the desired diacetate (14i) 1.12 g, 4.5 mmol, 62%, mp 106-107°. IR (KBr) 1780 cm⁻¹ (C=O). ¹H NMR 2.18 (3H, s), 2.38 (3H, s) 7.59 (2H, m), 8.38 (1H, s), 8.56 (1H, m).

3-Hydroxy-2-(carboxaldehyde)pyridine O-benzyloxime acetate (14ii): 3-Hydroxy-2-(carboxaldehyde)pyridine O-benzyloxime (13ii) (228 mg, 1 mmol), acetic anhydride (10 mL) and acetyl chloride (1 drop) were heated at reflux for 6 hr. The reaction mixture was concentrated in vacuo to remove excess acetic anhydride and water (10 mL) was added to the residue. The aqueous solution was neutralized with sodium carbonate and the resulting solution extracted with chloroform. The chloroform solution was dried over sodium sulfate and evaporated to dryness. The residue was subjected to preparative tlc (EtOAc, silica gel) to yield 277 mg, 0.97 mmol, 97% of the desired acetate (oil), IR (neat) 1780 cm⁻¹ (C=O). ¹H NMR 2.18 (3H, s), 5.26 (2H, s), 7.38 (6H, m), 8.30 (1H, s), 8.50 (1H, m).

Attempted debenzoylation of 14ii: PRESUMED 3-hydroxy-2-aminomethylpyridyl acetamide. To a solution of 14ii (100 mg, 0.34 mmol) in absolute ethanol (10 mL) there was added 10% Pd/C (100 mg). After stirring under an atmosphere of hydrogen for one hr at room temperature, the catalyst was removed by filtration and the filtrate evaporated to dryness at reduced pressure. The title amide resulted as a pale yellow low melting solid IR (KBr) 1640 cm⁻¹ (C=O), mp 137-138°. ¹H NMR 2.05 (3H, s), 4.77 (2H, d), 7.25 (2H, m), 8.00 (1H, m), 8.75 (1H, m).

3-Acetoxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime (14iii): A mixture of 3-hydroxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime, 13iii (88.2 mg, 0.35 mmol) in acetic anhydride (2.0 mL) and triethylamine (0.49 mL) and one drop of acetyl chloride was stirred at room temperature under nitrogen for two hr. The solvent was removed at reduced pressure and the residue was dissolved in chloroform (5 mL). Preparative tlc (ether/hexane 1:1) yielded 3-acetoxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime (14iii), 44.7 mg, (0.15 mmol, 47%). IR (KBr) 1790 cm⁻¹. ¹H NMR 0.98 (9H, s), 2.30 (3H, s), 7.30 (2H, m) 8.32 (1H, s), 8.50 (1H, m). And 3-acetoxy-2-(carboxaldehyde pyridine oxime (8.2 mg), mp 102-105. IR (KBr) 1780 cm⁻¹ (C=O). ¹H NMR 1.10 (3H, s), 7.30 (2H, m), 8.21 (1H, m), 8.58 (1H, s). Attempted methylation (methyl iodide/acetone) led to recovery of starting material; 3-acetoxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime methiodide (15i) was not isolated.

3-Acetoxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime methiodide (15i): (a) 3-Hydroxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime methiodide: 3-Hydroxy-2-(carboxaldehyde)pyridine t-butyldimethylsilyl oxime (13iii) (63.6 mg, 0.25 mmol) was dissolved in acetone (10 mL) with stirring. An excess of methyl iodide (2.5 mL, 24 mmol) was added and the reaction mixture warmed on the steam bath. The progress of the reaction was monitored by tlc (1:1 hexane/ether). When the starting material was consumed, the solvent and unreacted methyl iodide was removed at reduced pressure. The residue crystallized to yield 67 mg, 0.17 mmol, 68%, mp 260° (dec) of presumed methiodide. (b) The above formed methiodide was treated with an excess of acetic anhydride and trimethylamine. After standing overnight at room temperature, the solvent was removed to provide the corresponding acetate, mp 87° IR (KBr) 1740 cm⁻¹

Discussion:

Series b - Scheme 2

Initially, ethyl 2-methylnicotinate (16), prepared by the reaction of acrolein with ethyl 2-aminocrotonate (vide supra) was decided upon as a starting material because it was believed that the corresponding 2-PAM derivative might be synthesized directly. As already indicated, the unexpected intrusion to yield the heterocycle 2 frustrated that anticipation and, vide infra, development of an alternative was necessary.

Thus, the ester 16 was oxidized by (among others) m-chloroperbenzoic acid to the corresponding N-oxide and the N-oxide induced to undergo rearrangement in acetic anhydride to the corresponding ethyl 2-(acetoxymethyl)nicotinate, i.e. 17 → 18. Repetition of this sequence of oxidation and rearrangement thus led, via the N-oxide 19 to the diacetate of the desired aldehyde, i.e., 20. As we anticipated that hydrolysis of 20 would lead to a lactone, the triester (20) was treated with hydroxylamine directly. Rapid loss of the ethyl ester was obvious from the spectra and no evidence for oxime formation could be adduced. Spectroscopic evidence is in concert with formulation of the product as 2.

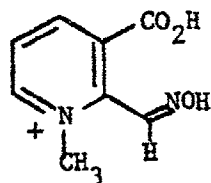
During this period, having faced difficulties in the attempted reaction between acrolein and 2-aminocrotonamide (vide supra) the amine series proposed for synthesis was examined. Thus, the ester 16 was converted to the N,N-dimethylamide (21) and the oxidation to the N-oxide (22) followed by rearrangement to the amidoester (23) was effected. Further examination in this series has not yet continued although a second oxidation and elaboration as above (but without cyclization) is anticipated.

It was early recognized that amino functionality might be introduced by utilization of the oxazolino substituent. Thus, although this was not part of the original proposal, we believed that exploration of some oxazoline chemistry in this series might serve two purposes. First, as already indicated, amino functionality would be present and, quaternization of this amino group would provide the electronic activation desired. Secondly, it was clear the oxazoline could be considered as a protecting group for the carboxylic acid and, should oxime formation succeed, it might be removed to provide the desired nicotinic acid derivative itself. Thus, the ethyl ester (16) was allowed to react with 2-amino-2-methyl-1-propanol to generate the 2-methyl-3-oxazoline 24 which, as now expected, was converted to the corresponding pyridine N-oxide (25) with m-chloroperbenzoic acid and thence to the ester (26). Again, oxidation to the oxide 29 and rearrangement to the diester (30) was successful. However, hydrolysis of 30 to the aldehyde 28 has not yet succeeded. The aldehyde precursor to the oxime desired (i.e., 28) has, in the meantime been prepared via hydrolysis of the monoacetate 26 to the carbinol 27 and oxidation with barium manganate. The yield in this last step is, however, poor and further work is needed. The oxime has not yet been generated.

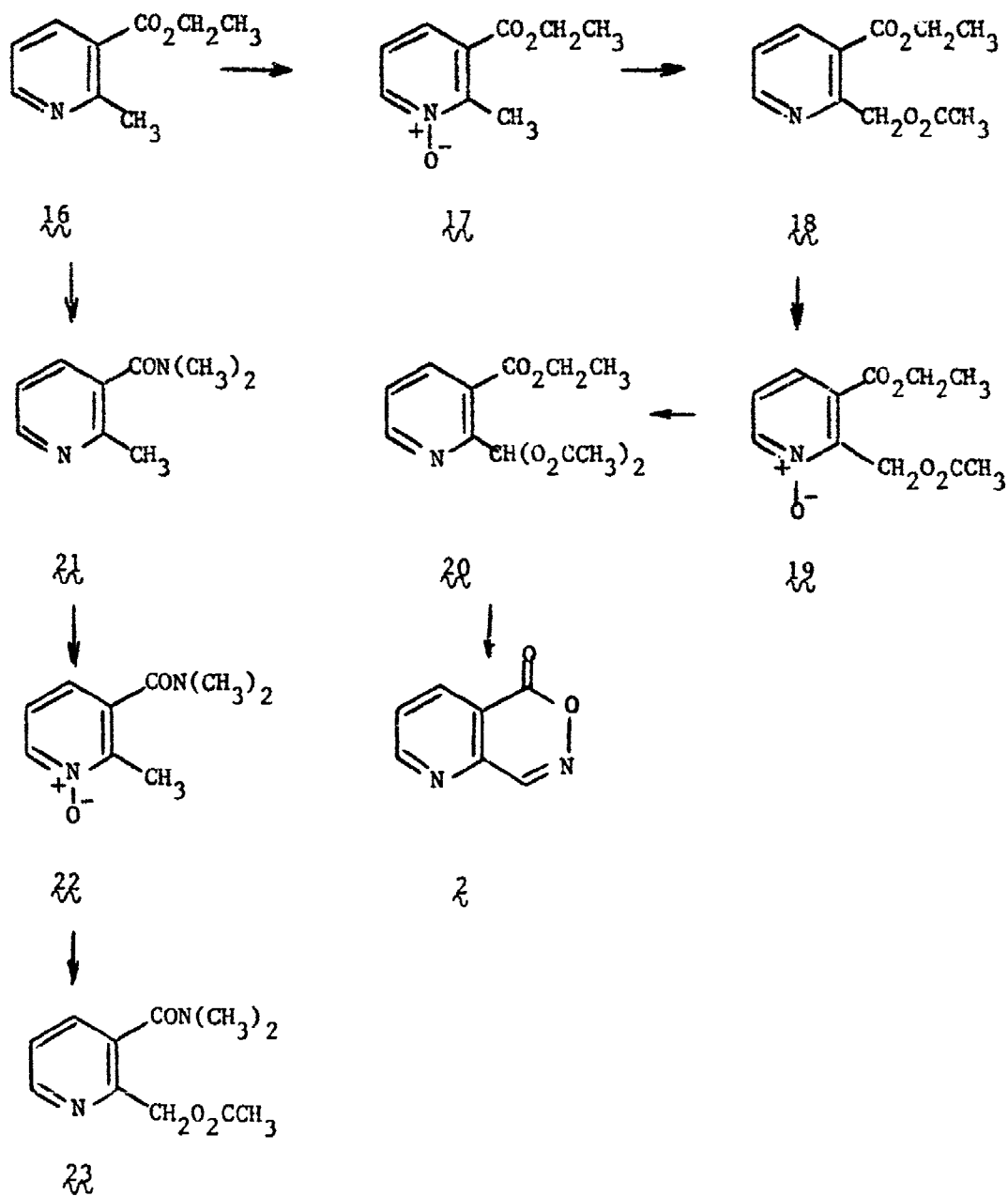
In the meantime, recognizing the difficulty with the ethyl ester 16 outlined above (i.e., intramolecular cyclization to 2) it was decided that the corresponding t-butyl ester, typical of its kind, would almost certainly be resistant to self-destruction and would have the added advantage of being susceptible to acid cleavage to form the desired salts. Therefore,

t-butyl 2-methylnicotinate, 31, was prepared by the reaction between acrolein, 5, and t-butyl aminocrotonate, 6 ($L = CO_2C(CH_3)_3$) - the latter having been synthesized by treatment of t-butyl acetoacetate with aq. ammonia. In the usual way, oxidation with m-chloroperbenzoic acid to the N-oxide 32, rearrangement to the acetate 33, oxidation again to the N-oxide 34 and a second rearrangement to the diacetate 35 succeeded. Then, careful acidic hydrolysis to the desired aldehyde 36 could be effected. Formation of the oxime 37 and methylation to the corresponding methiodide 38 was uneventful. Gram quantities of this methiodide (38) are on hand. Indeed, utilizing aqueous hydrogen iodide, hydrolysis to the acid 41, needed for preparation of the metal salts to be submitted, has also been effected and, in good yield, reaction of this acid with calcium carbonate, has generated a very hygroscopic material presumed to be the desired calcium salt. However, this was not pursued when we were informed (by the COTR) that the iodide salt was not desired. Hence, the methiodide ester 38, was, in aqueous solution, treated with IRA-900 ion exchange resin (chloride form) in an attempt to effect exchange of chloride for iodide. Only a low (ca 25%) yield of the corresponding acid (!) 40 could be isolated and extraction of the resin proved unsuccessful. However, use of IRA-958 resin (chloride form) has apparently succeeded in generating the chloride salt 39. We are awaiting elemental analysis before submission.

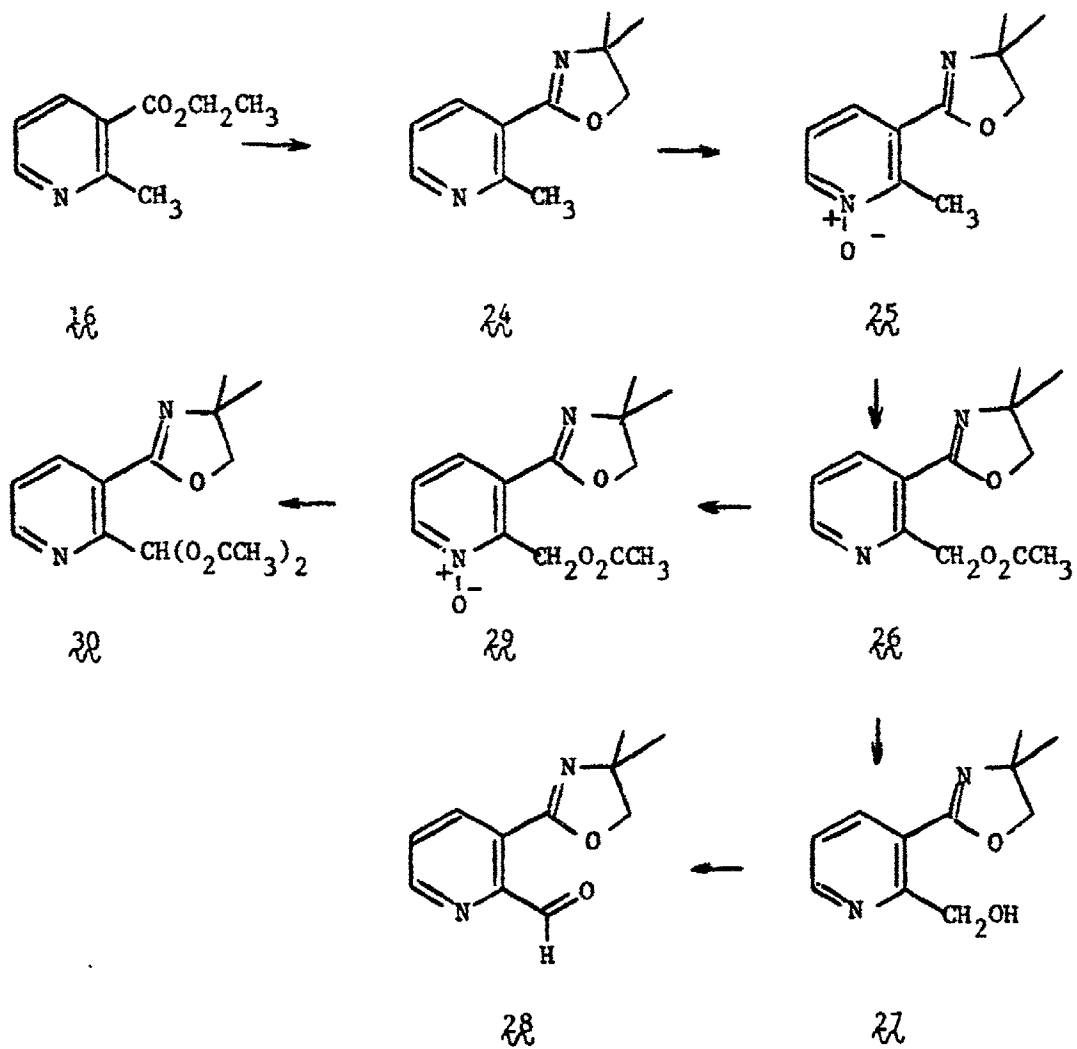
Series b



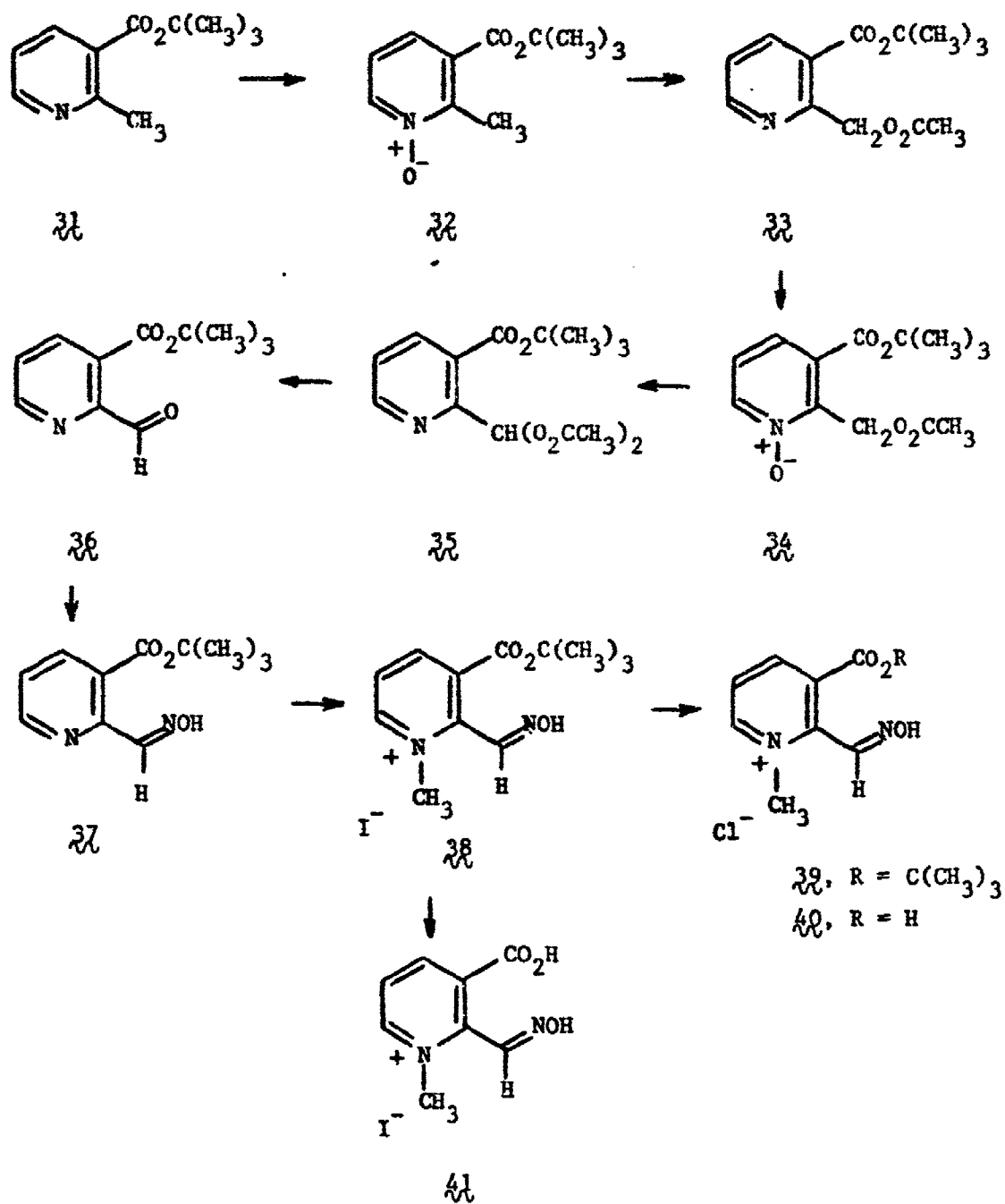
SCHEME 2



SCHEME 2 (continued)



SCHEME 2 (continued)



Experimental Section: Series b

Ethyl 2-methylnicotinate (16): METHOD A: To a mixture of ethyl 3-aminocrotonate (80 mL, 74.9 g, 0.58 mol), 2-propanol (140 mL) and piperidine (3 mL), which was stirred and held at 30° by cooling in an ice-water bath, was added acrolein (54 mL, 45.3 g, 0.81 mol) in the course of 2 hr. The progress of the reaction was monitored by GC (column 2% OV-17 on Chromsorb W, program 120-260 @ 20° min). The reaction was heated at reflux for 3.5 hr, after which the solvent was removed by simple distillation. To the residue, which was heated at 100°, powdered sulfur (61 g, 0.9 mol) was added portionwise. Subsequently, the reaction mixture, which fumed as a result of escaping hydrogen sulphide, was heated for 2 hr at 100° and then 1.5 hr at 125°. After cooling, chloroform (100 mL) was added and the sulfur removed by filtration. The chloroform solution was extracted with 2 N HCl (300 mL) and the aqueous solution backwashed with 3 x 100 mL fresh CHCl₃. The aqueous acid was then basified (with cooling) with 2 N NaOH and the ester so liberated extracted with chloroform (100 mL) and subsequently distilled in vacuo to obtain 46.4 g (48.5 %) of light yellow liquid (bp 115 - 130° @ 17 torr). IR (film) 1720 cm⁻¹ (C=O). ¹H NMR 8.5 (1H, dd), 8.1 (1H, dd), 7.06 (1H, dd), 4.38 (2H, q), 2.82 (3H, s), 1.4 (3H, t). ¹³C NMR 159.0, 150.8, 137.2, 125.1, 119.8, 60.2, 23.8, 13.5.

METHOD B: To a mixture of ethyl 3-amino crotonate (80 mL, 74.9 g, 0.58 mol), absolute ethanol (170 mL) and piperidine (3 mL), which was stirred and kept at 0°, was added acrolein (54 mL, 45.3 g, 0.81 mol) over 3 hr. After the addition was complete, the reaction mixture was heated at reflux for 24 hr. The solvent was then removed by simple distillation and the residue was subsequently distilled in vacuo to yield a mixture of ethyl 2-methylnicotinate and the corresponding dihydro derivative(s) (56.1 g, bp 70-150° @ 1 torr). The crude distillation mixture was oxidized by adding it to a mixture of concentrated sulfuric acid (32.7 g, 23.4 mL), concentrated nitric acid (35.1 g, 18.7 mL) and water (105 g, 105 mL). The resulting reaction mixture was cautiously warmed on a water bath with occasional stirring (HOOD) during which effervescence occurred over about 10 min. The resulting oxidation mixture was cooled to room temperature, extracted with ether (100 mL), made basic with saturated sodium bicarbonate and the basic solution extracted with ether again (5 x 50 mL). The combined ether extracts were dried over sodium sulfate, the ether removed by simple distillation and the residue distilled in vacuo to yield 28.9 g (30%) of the desired product (16) (bp 107° @ 10 torr) as a water white liquid which yellows on standing.

Ethyl 2-methylnicotinate N-oxide (17): To a stirred solution of ethyl 2-methylnicotinate (16) (330 mg, 2 mmol) in dichloromethane (30 mL) at 2° there was slowly added a solution of m-chloroperbenzoic acid (0.84 g, 4.87 mmol) in dichloromethane (10 mL). The resulting yellow solution was stirred at room temperature for 2 hr and then diluted with additional CH₂Cl₂ (100 mL), washed with 5% sodium carbonate (2 x 100 mL), and dried over anhydrous magnesium sulfate. After filtration and removal of the solvent at reduced pressure, the residue was chromatographed on silica gel (EtOAc) to yield 17 (315 mg, 1.74 mmol, 87%). IR (neat) 1720 cm⁻¹ (C=O). ¹H NMR 1.38 (3H, t), 2.72 (3H, s), 4.46 (2H, q), 7.18 (1H, dd), 7.65 (1H, d), 8.34 (1H, d). ¹³C NMR 13.8, 14.4, 61.7, 117.9, 121.7, 125.7, 130.1, 141.2.

Ethyl 2-(acetoxymethyl)nicotinate (18): The crude ethyl 2-methyl-nicotinate N-oxide (17) (2.23 g, 10 mmol) in acetic anhydride (10 mL) was added slowly to gently boiling acetic anhydride (30 mL). The reaction was then refluxed for 30 min after the addition was complete and then concentrated in vacuo, with heating, to remove excess acetic anhydride. Water (50 mL) was added to the residue which was then neutralized with sodium carbonate and extracted with chloroform (50 mL). The chloroform solution was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel (EtOAc) to give 18 (1.25 g, 5.6 mmol, 56%). IR (film) 1720 cm^{-1} (C=O). ^1H NMR 1.36 (3H, t), 2.19 (3H, s), 4.36 (2H, q), 5.56 (2H, s), 7.25 (1H, dd), 8.18 (1H, dd), 8.63 (1H, dd). ^{13}C NMR 13.79, 20.23, 61.16, 65.41, 122.07, 125.41, 137.97, 151.43, 155.92, 165.26, 169.86.

Ethyl 2-(acetoxymethyl)nicotinate N-oxide (19): To a stirred solution of ethyl 2-(acetoxymethyl)nicotinate (18) (446 mg, 2 mmol) in dichloromethane (30 mL) was slowly added a solution of m-chloroperbenzoic acid (840 mg, 4.87 mmol) in dichloromethane (10 mL). The resulting yellow solution was stirred at room temperature for 2 hr and then diluted with additional CH_2Cl_2 (100 mL), washed with 5% sodium carbonate solution (2 x 100 mL), and dried over anhydrous magnesium sulfate. After filtration and removal of solvent at reduced pressure, the residue was chromatographed on silica gel (EtOAc: CHCl_3 ; 1:1) to obtain 19 (410 mg, 1.7 mmol, 85%). ^1H NMR 1.38 (3H, t), 2.03 (3H, s), 4.38 (2H, q), 5.68 (2H, s), 7.26 (1H, m), 7.58 (1H, m), 8.30 (1H, m).

Ethyl 2-(bisacetoxymethyl)nicotinate (20): The N-oxide, 19, (2.39 g, 10 mmol) was treated with acetic anhydride (30 mL), as before, to effect rearrangement. The mixture was heated at reflux for 30 min and, after concentration in vacuo, to remove excess acetic anhydride, water was added to the residue, the aqueous solution neutralized with aqueous sodium carbonate, and extracted with chloroform (50 mL). The chloroform solution was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel (EtOAc: CHCl_3 ; 1:1) to obtain 20 (1.42 g, 5 mmol, 50%). IR (neat) 1718 cm^{-1} , ^1H NMR 1.39 (3H, t), 2.13 (6H, s), 4.40 (2H, q), 7.38 (1H, dd), 8.25 (1H, dd), 8.30 (1H, s), 8.75 (1H, dd). ^{13}C NMR 13.9, 20.2, 20.3, 61.8, 87.6, 117.9, 123.5, 138.4.

2-Methyl-N,N-dimethylnicotinamide (21): Ethyl 3-methylnicotinate (16) (660 mg, 2 mmol) was dissolved in 40% aq. dimethylamine (40 mL) and the mixture stirred for 72 hr at room temperature. The excess dimethylamine was removed under reduced pressure without heating and the remaining aqueous solution was continuously extracted with chloroform. The chloroform extract was concentrated in vacuo to obtain the amide 21 (132 mg, 0.8 mmol, 40%). IR (neat) 1618 cm^{-1} (C=O). ^1H NMR 2.5 (3H, s), 2.85 (3H, s), 3.12 (3H, s), 7.16 (1H, dd), 7.25 (1H, dd), 8.48 (1H, dd). ^{13}C NMR 21.4, 33.9, 37.5, 120.1, 131.4, 133.1, 148.6, 153.7.

2-Methyl-N,N-dimethylnicotinamide N-oxide (22): 2-Methyl-N,N-dimethylnicotinamide (21) (318 mg, 2 mmol) in dichloromethane (30 mL) at 2°C was treated, with stirring, with a solution of m-chloroperbenzoic acid (840 mg, 4.87 mmol) in dichloromethane (10 mL). The resulting yellow

solution was stirred at room temperature for 2 hr and then diluted with additional dichloromethane (100 mL), washed with 5% sodium carbonate solution (2 x 100 mL), and evaporated to dryness (after drying). As only a small amount of the desired N-oxide was found, the aqueous solution was continuously extracted with chloroform and, after drying over sodium sulfate, the chloroform was removed at reduced pressure to obtain the N-oxide 22, mp 123-125° (306 mg, 1.7 mmol, 85%). IR (KBr) 1630 cm⁻¹ (C=O). ¹H NMR 2.43 (3H, s), 2.87 (3H, s), 3.11 (3H, s), 8.18 (2H, m), 8.23 (1H, dd). ¹³C NMR 13.9, 34.1, 37.7, 121.7, 122.7, 135.1, 138.6, 145.5.

2-(Acetoxymethyl)-N,N-dimethylnicotinamide (23): The N-oxide (22), (360 mg, 2 mmol) was treated with acetic anhydride (10 mL) as before to effect rearrangement. The mixture was heated at reflux for 30 min. After concentration in vacuo to remove excess acetic anhydride, water was added to the residue, the aqueous solution neutralized with solid sodium carbonate, and extracted with chloroform. The chloroform solution was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on silica gel (1:1 ethyl acetate:chloroform) to obtain 23 (281 mg, 1 mmol, 50%). IR (neat) C=O at 1760 and 1640 cm⁻¹, ¹H NMR 2.08 (3H, s), 2.86 (3H, s), 3.10 (3H, s), 5.21 (2H, s), 7.21 (1H, dd), 7.56 (1H, dd), 8.59 (1H, dd). ¹³C NMR 20.5, 34.7, 38.6, 65.2, 122.4, 131.8, 134.3, 149.5, 152.3, 168.5, 170.0.

2-Methyl-3-(4,4-dimethyloxazolin-2-yl)pyridine (24): Ethyl 2-methylnicotinate (16), 25 g, 151.5 mmol) was heated, at reflux with 2-amino-2-methylpropan-1-ol (26.7 g, 300 mmol) for 10 hr. The reaction mixture was distilled in vacuo to obtain 24 (24.5 g, 129 mmol, 85%) bp 90-100° @ 17 torr. IR (neat) 1640 cm⁻¹ (C=N). ¹H NMR 1.34 (6H, s), 2.82 (3H, s), 4.02 (2H, s), 7.07 (1H, dd), 8.00 (1H, dd), 8.47 (1H, dd). ¹³C NMR 24.0, 27.6, 67.7, 77.9, 119.6, 122.7, 136.3, 148.5, 158.0, 160.1.

2-Acetoxymethyl-3-(4,4-dimethyloxazolin-2-yl)pyridine (26): The oxazoline 24, (0.95 g, 5 mmol) in dichloromethane (30 mL) was stirred at 2° while there was slowly added to it a solution of m-chloroperbenzoic acid (862.5 mg, 5 mmol) in dichloromethane (10 mL). The crude N-oxide (25) was worked up as indicated above and, without further purification, 25, in acetic anhydride (10 mL) was added dropwise to an additional portion (30 mL) of acetic anhydride at reflux. The mixture was heated at reflux for another 30 min and was worked up as indicated above to yield 26 (806 mg, 3.25 mmol, 65%) IR (neat) 1765 cm⁻¹ (C=O). ¹H NMR 1.32 (6H, s), 2.10 (3H, s), 4.02 (2H, s), 5.58 (2H, s), 7.20 (1H, dd), 8.10 (1H, dd), 8.52 (1H, dd). ¹³C NMR 20.3, 27.8, 65.5, 68.0, 78.6, 121.8, 122.8, 137.1, 150.7, 154.8, 159.4, 169.9.

2-(Hydroxymethyl)-3-(4,4-dimethyloxazolin-2-yl)pyridine (27): Potassium hydroxide (250 mg, 4.45 mmol) was dissolved in ethanol (95%, 10 mL) and this solution was added to a solution of 2-acetoxymethyl-3-(4,4-dimethyloxazolin-2-yl)pyridine (26) (270 mg, 1.09 mmol) in ethanol (95%, 5 mL). The mixture was heated on the steam bath for 3 hr. After removal of the ethanol at reduced pressure, the residue was dissolved in water (20 mL) and neutralized with dilute aqueous HCl. The neutral solution was continuously extracted with chloroform for 12 hr and the organic extract dried over sodium sulfate, filtered, and concentrated at reduced pressure to give a yellow oil, identified as the desired alcohol (170 mg, 0.83 mmol 76%). IR 1675 (C=N). ¹H NMR 1.28 (6H, s), 4.00 (2H, s), 4.84 (2H, s), 5.60 (1H, bs), 7.17 (1H, dd), 8.03 (1H, dd), 8.48 (1H, dd). ¹³C NMR 28.2, 65.2, 68.2, 78.8, 121.8, 122.1, 137.5, 149.9, 150.3.

3-(4,4-Dimethyloxazolin-2-yl)pyridine 2-carboxaldehyde (28): To a magnetically stirred solution of 2-(hydroxymethyl)-3-(4,4-dimethyloxazolin-2-yl)pyridine, 27 (170 mg, 0.83 mmol) in methylene chloride (20 mL) was added dry powdered barium manganate (1.68 g, 7.14 mmol) and the reaction mixture was heated to reflux. The progress of the reaction was followed by gc and was essentially complete in 12 hr. The cooled reaction mixture was diluted with methylene chloride (20 mL) and filtered through a celite mat. The filter cake was washed twice with methylene chloride (20 mL) with stirring and the filtrate combined with the washings. The combined methylene chloride phases were removed at reduced pressure to afford the desired aldehyde, 28 (62.0 mg, 0.30 mmol, 36.8 %). IR (neat) 1720 cm^{-1} (C=O). ^1H NMR 1.40 (6H, s), 4.18 (2H, s), 7.46 (1H, dd), 8.13 (1H, m), 8.80 (1H, m). ^{13}C NMR 28.0, 68.6, 79.8, 125.8, 137.4, 137.9, 151.1, 151.9, 159.5, 191.2.

3-(4,4-Dimethyloxazolin-2-yl)-2-(bisacetoxymethyl)pyridine (30): To a stirred solution of 2-acetoxymethyl-3-(4,4-dimethyloxazolin-2-yl)pyridine, 26, (15 g, 60 mmol) in dichloromethane (200 mL) at 2° there was slowly added a solution of *m*-chloroperbenzoic acid (15.5 g, 90 mmol) in dichloromethane (100 mL). The crude N-oxide was worked up in the usual way and, without further purification, it was treated with acetic anhydride (20 mL) and added dropwise to refluxing acetic anhydride (30 mL). The resulting reaction mixture was heated at reflux for another 30 min and, after cooling to room temperature, was worked up as usual. The residue was chromatographed on silica gel (EtOAc) to obtain the desired diacetate (7.71 g, 25.3 mmol, 42%) IR (neat) 1780 cm^{-1}

t-Butyl 2-methylnicotinate (31): t-Butyl aminocrotonate (prepared by allowing t-butyl acetoacetate to react with ammonium hydroxide in ethanol) (157 g, 1 mol) in benzene (500 mL) cooled to 2° in an ice-water bath was stirred while acrolein (1 mol, 67 mL) was added dropwise over 2 hr. The solution was heated to reflux overnight and then fitted with an air bubbler and air was bubbled through the warm solution for three (3) days. The benzene was removed at reduced pressure and the residue was distilled in vacuo. The ester, 31, bp $90-92^\circ$ @ 1 mm, 10%, was collected as a water white liquid. IR (neat) 1735 cm^{-1} (C=O)

t-Butyl 2-(acetoxymethyl)nicotinate (33): To a stirred solution of t-butyl 2-methylnicotinate (31) (9.65 g, 50 mmol) in dichloromethane (500 mL) at 2° there was slowly added a solution of *m*-chloroperbenzoic acid (21.6 g, 125 mmol) in dichloromethane (200 mL). The resulting yellow solution was stirred at room temperature for 4 hr and then washed with 5% sodium carbonate (4 x 100 mL) and dried over anhydrous magnesium sulfate. After filtration and removal of solvent, the crude N-oxide residue in acetic anhydride (20 mL) was added dropwise to acetic anhydride (60 mL) which was heated to reflux during the addition. After the addition was complete, the solution was refluxed for an additional 30 min and then concentrated at reduced pressure to remove excess acetic anhydride. Water (50 mL) was added to the residue and the resulting solution neutralized with sodium carbonate (solid) and extracted with chloroform (4 x 50 mL). The combined chloroform extracts were evaporated to dryness and chromatographed on silica gel (EtOAc) to give 33, 7.53g, 30 mmol, 60%. IR (neat) 1725 cm^{-1} (C=O). ^1H NMR 1.55 (9H, s), 2.12 (3H, s), 5.55 (2H, s), 7.25 (1H, dd), 8.13 (1H, dd), 8.63 (1H, dd). ^{13}C NMR 20.3, 27.7, 65.5, 82.1, 121.9, 126.8, 137.9, 150.8, 155.4, 164.5, 169.9.

t-Butyl 2-(bisacetoxymethyl)nicotinate (35): To a stirred solution of t-butyl 2-acetoxymethylnicotinate (33) (7.53 g, 30 mmol) in dichloromethane (300 mL) at 20° there was slowly added a solution of m-chloroperbenzoic acid (13 g, 75 mmol) in dichloromethane (100 mL). The crude N-oxide was worked up as indicated above and, without purification, the residue, in acetic anhydride (20 mL), was added dropwise to refluxing acetic anhydride (40 mL). The reaction mixture was refluxed for another 30 min and, after concentration in vacuo to remove excess acetic anhydride, water was added to the residue. The aqueous solution was neutralized with aqueous sodium carbonate and extracted with chloroform (4 x 50 mL). The chloroform solution was dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel (eluting with 1:1 CHCl₃/EtOAc) to obtain 35 (3.84 g, 12.5 mmol, 41%). IR 1780 cm⁻¹ (C=O). ¹H NMR 1.67 (9H, s), 2.11 (3H, s), 7.35 (1H, dd), 8.17 (1H, dd), 8.30 (1H, s), 8.73 (1H, dd). ¹³C NMR 20.2, 17.7, 82.9, 123.4, 127.6, 138.2, 151.0, 152.6, 164.0, 168.2.

t-Butyl 2-(carboxaldehyde)nicotinate (36): Hydrochloric acid (3%, 2.4 mL) cooled to 0° was added to a solution of t-butyl 2-(bisacetoxymethyl)nicotinate (35) (0.125 g, 0.405 mmol) in acetone (1 mL). The reaction mixture was stirred at room temperature for 28 hr. After neutralization with saturated bicarbonate solution, the aqueous mixture was extracted with ether (3 x 15 mL). The combined ether extracts were dried over sodium sulfate, filtered and concentrated at reduced pressure (WITHOUT HEATING) to give a pale yellow oil which was identified as the desired aldehyde (52 mg, 0.251 mmol, 62%) by ¹H NMR. (IR, neat 1740 cm⁻¹) 10.3 (1H, s), 8.8, 8.1, 7.5 (3H), 1.65 (9H, s). The aldehyde was used directly to prepare the corresponding oxime without further purification.

t-Butyl 2-carboxaldehydeoxime nicotinate (37): To a solution of crude t-butyl 2-(carboxaldehyde)nicotinate (36) (52 mg, 0.25 mmol) in 95% ethanol (1.0 mL) was added an aqueous solution (1 mL) of hydroxylamine hydrochloride (30 mg, 0.432 mmol) and sodium acetate trihydrate (30 mg, 0.22 mmol). The reaction mixture was stirred at room temperature for 30 min and then heated on a steam bath for another 30 min. The reaction mixture was then diluted with water (3 mL) and then extracted with warm chloroform (3 x 5 mL) and the combined chloroform extracts dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to a purple residue. Passage of a solution of this residue (in chloroform) through a small plug of silica gel followed by further elution with ether yielded a pale yellow oil which solidified on standing. Recrystallization from benzene/cyclohexane (1:1) yielded white crystals (mp 141-142°) of the desired oxime (48 mg, 0.22 mmol, 86%). IR (KBr) 3600-2600 cm⁻¹ (OH), 1730 cm⁻¹ (C=O). ¹H NMR 8.9 (1H, s), 8.8, 8.1, 7.3 (3H), 1.6 (9H, s). ¹³C NMR 164.9, 151.7, 150.4, 148.1, 138.1, 127.9, 123.2, 83.0, 28.1.

t-Butyl 2-carboxaldehyde oxime nicotinate methiodide (38): To a solution of t-butyl 2-carboxaldehyde oxime nicotinate (37) (0.5 g, 2.26 mmol) in acetone (5 mL) was added excess iodomethane (6 mL) and the reaction mixture heated at 40° for 36 hr. The reaction mixture was concentrated at reduced pressure to give a reddish-brown residue. The residue was dissolved in the minimum amount of 1:1 benzene/ethanol and ether added to induce precipitation. Crystallization was effected from benzene-ethanol to afford pale yellow crystals of the desired methiodide (0.57 g, 70%) mp 143-144 (dec.) IR (KBr) 3600-2500 (-OH). ¹H NMR (acetone-d₆) 1.6 (9H, s), 4.7 (3H, s), 8.3-9.6 (3H), 8.8 (1H, s).

t-Butyl 2-carboxaldehyde oxime nicotinate methochloride (39): Incomplete as of 31 July 1986. The methiodide 38 (50 mg, ca 0.1 M) in water is passed through IRA-958 resin - chloride form (Rohm and Haas Co.) which has been pretreated with dil. aq. NaOH, dil. aq. NaCl, and extensively washed with deionized, distilled water. The t-butyl ester 39 (41 mg) is recovered. The use of IRA-900/Cl⁻ form results in ester hydrolysis to yield 40. Thus, a 0.08 N solution of the methiodide 38 (0.61 g in 21 mL of HPLC grade water) was passed through an ion exchange resin (10 mL, Amberlite IRA-900/Cl⁻). Ten fractions (6 mL each) were collected and the water removed at reduced pressure to give a pale yellow solid. Recrystallization from ethanol/water yielded a white solid (75 mg) mp 202-203° (dec). Efforts to remove additional material from the column by washing with water or acetic acid/water were unsuccessful. The material is assigned the structure of the carboxylic acid 40. ¹H NMR (D₂O) 4.2 (3H, s), 8.0-8.8 (3H), 8.5 (1H, s). Initial efforts indicate that treatment of methiodide 38 with aqueous HI generates 41. Thus, by analogy, t-butyl 2-methylnicotinate (31) (0.8 g, 4.15 mmol) in acetone (5 mL) was treated with excess iodomethane (5 mL) and the mixture heated at reflux on the steam bath for 12 hr. The mixture was concentrated at reduced pressure and the resulting reddish-brown residue crystallized. Recrystallization from methanol (by addition of ether) gave pale yellow crystals (mp 139-140) of the corresponding methiodide (1.25 g, 3.73 mmol, 95%). IR (KBr) 1730 cm⁻¹. ¹H NMR (acetone-d₆) 1.6 (9H, s), 3.1 (3H, s), 4.6 (3H, s), 8.1 - 9.4 (3H). The methiodide so prepared (1.0 g, 2.99 mmol) in water was treated with an excess of aq. HI (2 mL) and the mixture stirred at room temperature for 6 hr. The reaction mixture was filtered and the filtrate concentrated at reduced pressure. The brown residue was recrystallized from 95% ethanol to yield off-white crystals (mp 224°) of the desired acid (0.59 g, 2.2 mmol, 71 % of theory). IR (KBr) 3600-2600 cm⁻¹ and 1730 cm⁻¹ (CO₂H). ¹H NMR (dmsd-d₆) 2.9 (3H, s), 4.3 (3H, s), 8.0-9.1 (3H). The corresponding calcium salt of this carboxylic acid was prepared as follows: To a solution of the acid methiodide (0.15 g, 0.54 mmol) in water (3 mL) was added anhydrous calcium carbonate (27 mg, 0.27 mmol) and the reaction mixture heated on a steam bath for 30 min and then stirred at room temperature for 1 hr. The reaction mixture was filtered and the filtrate concentrated at reduced pressure to give a pale yellow residue. Recrystallization was effected from 95% ethanol to give off-white crystals (153 mg, 0.26 mmol, 95% of theory). IR (KBr) 3600-3150 cm⁻¹ (CO₂⁻), 1660 and 1560 cm⁻¹. ¹H NMR (D₂O) 2.9 (3H, s), 4.3 (3H, s) 7.9 -8.7 (3H).

Discussion:

Series c - Scheme 3:

As indicated in Scheme 2 (vide supra) ethyl 2-methylnicotinate (16) was prepared by the reaction between acrolein (5) and ethyl aminocrotonate (6, L = CO₂Et). As anticipated, the ethyl ester was cleanly reduced with lithium aluminum hydride in ether to the corresponding alcohol 42 and the latter converted, via the corresponding chloride (43) to the nitrile 45. Treatment of the nitrile with ethanol and gaseous hydrogen chloride generated the ethyl ester 46, (R = OEt). The corresponding methyl ester (46, R = OMe) was also made by substitution of methanol for ethanol. Neither the benzyl ester nor the *t*-butyl ester could be prepared (as was not unexpected) by this method. The amide 46 (R = NH₂) was also prepared by partial hydrolysis of the nitrile 45.

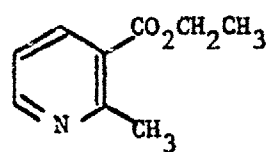
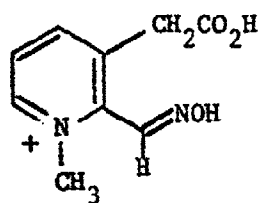
The N-oxide 47 was prepared by *m*-chloroperbenzoic acid oxidation of the ester 46 (R = OEt) and, in the usual fashion, rearrangement to the diester 48 effected. Further oxidation to 49 and rearrangement to 50 and hydrolysis with dilute acid then generated the aldehyde 51. Without event, the oxime 52 and the corresponding methiodide 53 (submitted as BL 19566) were formed.

In addition to the work outlined above, some efforts were expended in attempting to reduce the nitrile 45 and the amide 46 (R = NH₂) to the corresponding amine. Lithium aluminum hydride, under a wide variety of conditions, was unsuccessful. Some reduction could, however, be effected with hydrogen in the presence of platinum oxide. This has not yet been pursued extensively.

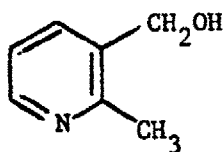
As an aside, the *t*-butyl ester (46, R = O-*t*Bu) was prepared by saponification of the ethyl ester 46 (R = OEt) to the corresponding acid, conversion to the acid chloride 46 (R = Cl) with thionyl chloride, and treatment of the latter with *t*-butanol. As we have shown that the *t*-butyl ester (vide supra) of the methiodide oximes can be cleaved with hydrogen iodide and that ion exchange is successful, this allows formation of salts in this series too.

Series c

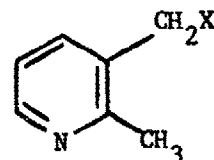
SCHEME 3



16

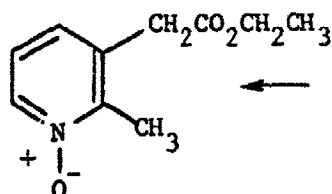


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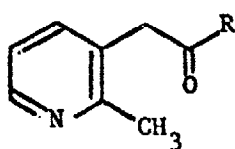


43, X = Cl

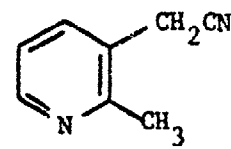
44, X = CH2NH2



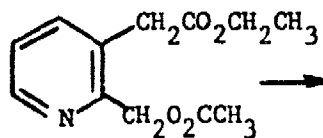
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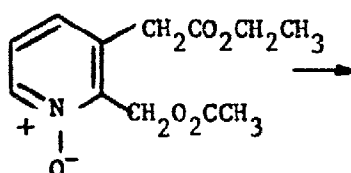
46, R = OEt, OMe, NH2



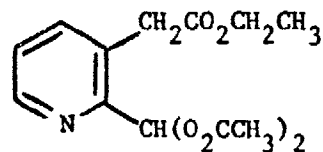
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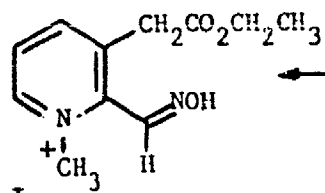
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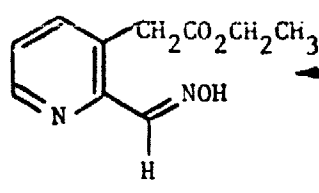
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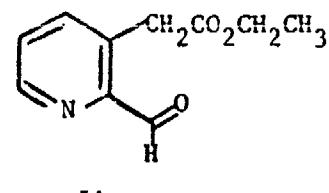
50



53



52



51

Experimental Section: Series c

2-Methyl-3-(hydroxymethyl)pyridine (42): A solution of ethyl 2-methyl-nicotinate (30 g, 181.8 mmol) in anhydrous ether (200 mL) was added dropwise to a stirred suspension of LiAlH_4 (9.0 g) in anhydrous ether (200 mL). The reaction mixture was refluxed for 3 hr under an atmosphere of N_2 , cooled in an ice bath and water cautiously added to destroy excess hydride. The suspension was filtered through a celite mat and the residue washed with hot ethanol (70 mL). The combined filtrates were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to give a pale yellow oil. Distillation in vacuo (bp 105-107 @ 1 torr) gave the desired alcohol (42) (18.0 g, 143.6 mmol, 80%). Alternatively, flash chromatography (silica gel, $\text{MeOH}/\text{CHCl}_3$: 1:10) yielded (fraction 1) 2,3-dimethylpyridine (230 mg) and fraction 2, 2-methyl-3-(hydroxymethyl)pyridine (42). IR (film 3600-3200 cm^{-1} (OH). ^1H NMR 8.2, 7.7, 7.0 (3H), 6.7 (1H, s), 4.6 (2H, s), 2.4 (3H, s). ^{13}C NMR 20.3, 60.3, 120.7, 134.4, 134.9, 146.0, 154.9.

3-Cyanomethyl-2-methylpyridine (45): A solution of 3-hydroxymethyl 2-methylpyridine (18.0 g, 146.3 mmol) in benzene (25 mL) was added dropwise into thionyl chloride (32.0 mL) with stirring and ice cooling and the mixture was then brought to room temperature and, finally, was heated at reflux for 2.5 hr. Excess thionyl chloride was removed under reduced pressure, the yellow residue dissolved in 60% ethanol (40 mL), and a solution of potassium cyanide (28.0 g) and potassium iodide (1.8 g) in 60% ethanol (175 mL) was added to the crude chloride solution (43), with stirring. The mixture was heated at reflux for 5 hr. On cooling, the precipitated salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in water (15 mL) and continuously extracted with CHCl_3 for 12 hr. The chloroform solution was dried over potassium carbonate, filtered through a plug of glass wool, and the solvent removed under reduced pressure to give a dark brown residue. Distillation in vacuo (bp 98-100° @ 1 torr) or flash chromatography on silica gel (EtOAc) yielded the title compound (14.3 g, 108.3 mmol, 74%). IR 2280 cm^{-1} (CN) ^1H NMR 8.3, 7.5, 7.1 (3H), 3.7 (2H, s), 2.4 (3H, s). ^{13}C NMR 155.8, 148.0, 135.3, 124.1, 121.1, 116.5, 21.5, 20.7.

Ethyl 2-methylpyridyl-3-acetate (46, R = OEt): Dry hydrogen chloride gas was introduced for 30 min into a solution of 3-cyanomethyl-2-methylpyridine (45) (7.5 g, 56.8 mmol) in a mixture of absolute ethanol (35 mL) and anhydrous ether (40 mL) with stirring and cooling (ice bath). The reaction mixture was refluxed for 5 hr during which time HCl gas was continuously passed through the solution. The salts deposited on cooling were filtered off and the solvent removed at reduced pressure. The residual oil was dissolved in water (10 mL), made alkaline with potassium carbonate (saturated solution) and continuously extracted with chloroform for 12 hr. The chloroform solution was dried over sodium sulfate, filtered, and the chloroform removed at reduced pressure to give an oily residue which, either on distillation in vacuo (bp 99-100° @ 1 torr) or on flash chromatography (silica gel, ethyl acetate) gave the title ester (8.70 g, 48.6 mmol, 86%) as a pale yellow oil. IR 1730 cm^{-1} (C=O). ^1H NMR 8.3, 7.4, 7.0 (3H), 4.1 (2H, q), 3.6 (2H, s), 2.5 (3H, s), 1.1 (3H, t). ^{13}C NMR 169.5, 156.6, 147.1, 127.7, 120.5, 60.0, 37.8, 21.6, 13.4. The corresponding methyl ester, 46 (R = OCH_3) was prepared similarly.

Ethyl 2-methylpyridyl-3-acetate N-oxide (47): To a solution of ethyl 2-methylpyridyl-3-acetate (46) (8.70 g, 48.6 mmol) in dichloromethane (200 mL) cooled in an ice bath, there was added, dropwise, with stirring, a solution of *m*-chloroperbenzoic acid (16.77 g, 97.2 mmol) in dichloromethane. After the addition was complete, the ice bath was removed and the mixture allowed to stir at room temperature for 5 hr. The reaction mixture was then stirred with an aqueous slurry of sodium carbonate and the precipitated salts removed by filtration through a celite mat. The filtrate was dried over anhydrous sodium carbonate, filtered, and the solvent removed under reduced pressure to give a yellow oil which, on flash chromatography (200 g silica gel methanol/chloroform 1:9) gave the desired N-oxide (7.62 g, 39.1 mmol, 81%) as a pale yellow oil which solidified on standing (mp 54-59°) IR (film) 1730 cm⁻¹ (C=O) ¹H NMR 8.3, 7.2 (1H), 4.1 (2H, q), 3.8 (2H, s), 2.5 (3H, s), 1.1 (3H, t).

Ethyl 2-(acetoxymethyl)pyridine-3-acetate (48) A solution of ethyl 2-methylpyridyl-3-acetate N-oxide (5.9 g, 30.3 mmol) in acetic anhydride (2 mL) was added dropwise to refluxing acetic anhydride (3 mL) and refluxing was continued for an additional 25 min. Excess acetic anhydride was removed at reduced pressure and the residue was dissolved in chloroform (200 mL). The CHCl₃ solution was stirred with an aqueous slurry of saturated sodium carbonate, the phases separated, the CHCl₃ dried by filtration, and the solvent removed in vacuo to yield an oily residue. Flash chromatography (200 g silica gel, ethyl acetate:chloroform 1:1) gave a mixture of the desired acetate, 48, and an isomer (presumably ethyl 5-acetoxy-2-methylpyridine-3-acetate) 5.21 g, which could not be separated by chromatography or by distillation (bp 146-148° @ 1 torr). (NOTE - only a minor effort was expended in examining separation at this point as it was quickly appreciated that such separation might more efficiently be affected subsequently. That this was the case can be seen (vide infra).

Ethyl 2-(bisacetoxymethyl)pyridine-3-acetate (50): *m*-Chloroperbenzoic acid (7.6 g, 43.9 mmol) in dichloromethane (75 mL) was added dropwise, with stirring, to a cold (ice-bath) solution of the crude mixture of ethyl 2-(acetoxymethyl)pyridine-3-acetate, 48, and (presumed) ethyl 5-acetoxy-2-methylpyridine-3-acetate (5.2 g) obtained above and dissolved in dichloromethane (75 mL). Stirring was continued after the addition was complete and while the solution warmed to room temperature for an additional 4 hr. The reaction mixture was diluted with additional dichloromethane (75 mL) and shaken with an aqueous slurry of saturated sodium carbonate. The dichloromethane was separated from the aqueous phase, filtered, and then removed under reduced pressure. The crude N-oxide (49 and its isomer) was dissolved in acetic anhydride (2 mL) and added dropwise into boiling acetic anhydride (3 mL). The reaction mixture was then refluxed for 20 min and the excess acetic anhydride removed at reduced pressure. Water (10 mL) was added to the residue which was then neutralized with solid sodium carbonate and extracted with chloroform (4 x 40 mL). The combined chloroform extracts were dried over sodium sulfate, filtered, and the solvent evaporated at reduced pressure to give an oil which was chromatographed (20 g silica gel, EtOAc/CHCl₃ 1:1). The diacetate (3.71 g, 12.6 mmol) was obtained as the second fraction. Small amounts of material corresponding to ethyl 5-acetoxy-2-methylpyridine-3-acetate, which had apparently not reacted to give either the N-oxide nor the subsequent acetate accompanied 50. IR (neat) 1720-1760 (C=O's).

Ethyl pyridyl-3-acetate-2-carboxaldehyde (51): Hydrochloric acid (13 mL, 6N) cooled to 0° was added in one portion to a solution of ethyl 2-(bisacetoxymethyl)pyridine-3-acetate (50) (0.7 g, 2.4 mmol) also cooled to 0° in an ice bath. The reaction mixture was stirred at 0° for 20 hr. After neutralization with solid sodium bicarbonate, the aqueous solution was extracted with ether (3 x 25 mL). The combined ether extracts were dried over sodium sulfate, filtered, and concentrated at reduced pressure to give a brown viscous oil. Passage of a solution of this oil (in ether) through a small plug of silica gel, followed by further elution with ether yielded the desired aldehyde (0.29 g, 1.49 mmol, 62%). IR (film) 1730 cm⁻¹ (C=O, ester), 1700 cm⁻¹ (C=O, aldehyde). ¹H NMR 10.2 (1H, s), 8.7, 7.6, 7.4 (3H), 4.2 (2H, q), 4.1 (2H, s), 1.1 (3H, t). ¹³C NMR 194.4, 169.6, 150.0, 148.2, 139.6, 130.9, 126.2, 60.5, 37.0, 13.7.

Ethyl pyridyl-3-acetate-2-carboxaldehyde oxime (52): To a solution of ethyl pyridyl-3-acetate-2-carboxaldehyde (51) (0.22 g, 1.14 mmol) in 95% ethanol (1.5 mL) was added hydroxylamine hydrochloride (0.174 g, 2.5 mmol) and pyridine (0.25 mL, 3.0 mmol). The reaction mixture was heated on a steam bath for 16 hr and then extracted with hot chloroform (5 x 2 mL). The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered, and concentrated at reduced pressure to give a tan solid. Passage of a solution of this material in chloroform through a small plug of silica gel followed by further elution with ether yielded a pale yellow oil which solidified on standing. Recrystallization from benzene/cyclohexane (1:1) yielded the oxime (0.20 g, 0.97 mmol, 85%) as white crystals, mp 127-128° IR (KBr) 3600-2600 cm⁻¹ (OH), 1730 cm⁻¹ (C=O). ¹H NMR 8.6, 7.6, 7.2 (3H), 8.4 (1H, s), 4.0 (2H, q), 3.9 (2H, s), 1.1 (3H, t). ¹³C NMR 170.4, 150.3, 149.8, 147.6, 139.2, 129.0, 122.7, 60.6, 38.9, 13.6.

Ethyl pyridyl-3-acetate-2-carboxaldehyde oxime methiodide (53): To a solution of ethyl pyridyl-3-acetate-2-carboxaldehyde oxime (52) (0.40 g, 1.92 mmol) in acetone (10 mL) was added excess methyl iodide (2.0 mL) and the mixture was heated gently on a steam bath for 5 hr during which time a pale yellow crystalline solid had separated. The solid was filtered off and the remaining solution examined by tlc (silica gel, ether) which showed unreacted starting material remained. The mother liquor was concentrated under reduced pressure, the residue dissolved in fresh acetone (5 mL) and an additional portion of methyl iodide added (2.0 mL). Heating at reflux for 2 hr yielded an additional crop of crystals, mp 197-199° (dec) identical to the first crop obtained. As starting material yet remained in the solution, the above process was repeated until all of the starting material had been consumed (tlc). The pale yellow solid thus obtained was combined with other batches and recrystallized from methanol to give 53 (0.61 g, 1.74 mmol, 91%). IR (KBr) 3600-2600 cm⁻¹ (OH), 1730 cm⁻¹ (C=O). ¹H NMR 9.1, 8.5, 8.1 (3H), 8.6 (1H, s), 4.4 (3H, s), 4.4 (2H, q), 4.1 (2H, s), 1.2 (3H, t). Calc. for C₁₁H₁₅O₃N₂I: C, 37.71; H, 4.29. Found C, 37.62; H, 4.42.

3-Acetamido-2-methylpyridine (46, R = NH₂): The nitrile 45 (2.64 g, 2.0 mmol) in t-butanol (20 mL) was treated with finely powdered KOH (4 g, 7.1 mmol) and the resulting solution heated at reflux for 35 min. After cooling, the reaction mixture was diluted with water (50 mL), added to an equal volume of saturated sodium chloride and continuously extracted with chloroform. Cooling the warm solution of chloroform yielded 1.93 g (64%) of the desired amide, mp 190-192° (lit¹⁶ 190-192°).

2-Amino-(2-methyl-3-pyridyl)ethane (44, X = CH₂NH₂): To the nitrile 45 (1.32 g, 10 mmol) dissolved in water (10 mL) in a Paar shaker bottle, was added PtO₂ (166.7 mg). The bottle was pressurized to 57 psi and shaken for 2 hr to reduce catalyst and saturate the solution with hydrogen (15 lb drop in pressure). Agitation was halted, the bottle repressurized to 57 psi and shaking was continued for an additional 12 hr. Catalyst was removed by filtration through a celite mat and the filtrate was made strongly basic by addition of 2 g. of powdered KOH pellets. The aqueous solution was then extracted with Et₂O/CCl₄ (1:1, 3 x 25 mL) and the combined organic phases dried over potassium carbonate. After filtration, the organic phase was taken to dryness at reduced pressure. Elution of a silica gel column with 10% methanol in chloroform yielded 739.6 mg (54.3%) of the desired oily amine 44. IR (neat) 3400 cm⁻¹ (NH). ¹H NMR 8.3, 7.4, 4.0 (3H), 2.8 (4H, bs), 2.54 (3H, s). ¹³C NMR 156.4, 146.5, 136.1, 132.9, 120.8, 49.2, 33.0, 21.9.

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